Chapter 11

Summary and Conclusions

A. The behaviour of *C. subterranea*

Individual *C. subterranea* were allowed to construct a 2-dimensional burrow inside sediment filled cuvettes. The cuvettes were tailored to their body width, enabling observation of the shrimps throughout their burrow. The shrimps were observed during continuous periods of 1 to 72 hrs. During observation periods longer than 2 hrs, several previously instructed observers worked in shifts. The behaviour of the shrimps was found to consist of 12 behavioural states, which were described in an ethogram: Sitting, Walking, Turning, Stirring up sediment, Lifting sediment, Carrying sediment, Dropping sediment, Bulldozering, Tamping the substrate, Ventilating, Pumping and Grooming. The behaviour was recorded manually using electronic event recorders with separate keys coding for the defined states. In total 273 hrs of protocol were recorded of 14 animals. Analysis of the resulting data showed that records of animals which were not visible for more than 10 % of the time significantly differed from records with 100 % visibility. In only 129 one-hour records of 13 animals, the shrimps had been visible for more than 90 % of the time. These records were rated representative for *C. subterranea*'s behaviour, and used for further analysis. A Markov-chain type sequence analysis showed that 18 sequences of behavioural states occurred significantly more often than expected stochastically. Based on these sequences, the 12 behavioural states were grouped to 6 functional classes of behaviour: Wandering, Burrowing, Surveying, Grooming, Resting and Ventilating. Frequencies and bout-lengths of these behavioural classes showed that *C. subterranea* on average Burrows every 7 min for 3 min followed by Wandering (17 s), Resting (1 min) or sometimes Surveying (1.3 min) or combinations of these. On average every 5 min the shrimp interrupts this pattern for a period of Grooming (46 s) and Ventilation of the burrow is on average performed every 14.5 min lasting about 1.3 min. This behavioural pattern is displayed constantly, Fourier analysis showed no significant periodicity. More than 40 % of the time that an animal is active (= not resting) is spent Burrowing, during which the animal is probably foraging as well. Pumping sediment to the sea floor surface is performed once or up to a few times per day. Since *C. subterranea*'s behaviour appeared to be very stereotype during the whole experimental period, we assume our results to reflect the natural behaviour of this shrimp at the Oyster Grounds closely.

B. The burrow architecture and turbative activity of *C. subterranea*

The architecture and development of the burrows of the endo-benthic shrimp *Callianassa subterranea* from the central North Sea was studied in mesocosms and sediment-filled cuvettes in the laboratory. In total 81 animals constructed a three-dimensional burrow. These were used to describe the general 3D burrow architecture. In total 41 animals made a two-dimensional burrow in cuvettes tailored to their body width. The development of 8 burrows in time was registered by regularly mapping the burrow outline and the sediment surface level. Excavation velocities and sediment expulsion rates in time were derived from changes in the burrow outlines and sediment surface levels on the maps. The total tunnel length increased at a rate of 23.4 ± 6.0 mm/hr during the the initial stage of burrow development. The length increase levelled off during the completion of the first and second U-tube to 5.6 ± 1.8 mm/hr. Initial sediment expulsion rates up to 15.0 cm^3/day were established. The average sediment expulsion rate was 1.080 ± 0.096 cm^3/day. Extrapolation to a yearly sediment turnover rate, including population density and water temperature effects, resulted in an estimate of 15.5 ± 2.7 kg dry mass per m^2 per year ($DM/m^2/yr$), a 1.2 cm layer. Samples of burrow lining, expelled sediment and unprocessed sediment did not show changes in grain size distributions or organic content due to animal handling. Burrow development experiments carried out in enriched seawater systems did not reveal consistent effects of particulate organic matter (POM) on sediment expulsion rates and on the composition of processed or unprocessed sediment.

C. Selective deposit feeding and its yield in *C. subterranea*

The trophic mode of *C. subterranea* can be characterized as genuine deposit feeding: *C. subterranea* feeds on sediment and processes it before ingestion. Sub-samples of sediment from the shrimps habitat and the stomach content were fractioned into 7 grain size classes. The stomach contained a significantly larger mass fraction of grains smaller than 30 µm, whereas the habitat sediment contained mainly grains larger than 70 µm. A more detailed look shows that grains smaller than 70 µm are significantly preferred by the shrimp, and larger grains are avoided. Analysis of the organic content of the habitat sediment, stomach content and the faecal pellets of *C. subterranea* showed a tenfold higher organic content of the stomach content compared to habitat sediment. This increase is mainly due to differences in total area of small

grains compared to large grains per unit of mass, assuming the same amount of organic matter per unit of area. The pellets contained half as much organic matter as the stomach content, indicating an digestion efficiency of about 50 %. The gut passage rate of *C. subterranea* was derived from the cumulative faecal production rate of 49 animals. These animals were put into quarantaine directly after they were removed from their burrows, and regularly checked on the production of faeces. The average faecal production stayed initially high, leveling of to almost zero after 2 hrs and staying low thereafter. Feeding of the shrimps after 12 hrs of quarantaine resulted in an increase in faecal production after another 2 hrs. From these results the average gut passage rate (*GPR*) was derived to be 2.38 ± 0.05 mm^3/hr, which could be converted to an average consumption of organic matter (*OCR*) of 4.2 ± 1.4 mgDM/day. From all data of the individual animals, relations of *GPR* and *OCR* vs. animal length resulted. A Length-Mass relation derived from 152 *C. subterranea* enabled conversion of the *OCR*-Length relation to a function relating *OCR* to the body ash-free dry mass ($AFDM_b$): $OCR_M = 0.133 * AFDM_b^{0.91}$, with $AFDM_b = 8.35 * 10^{-4} * L^{3.15}$ (OCR_M in mgDM/day, L in mm and $AFDM_b$ in mg).

D. Morphology, motion and function of the feeding appendages of *C. subterranea*

The morphology of the mouthparts of C. subterranea was studied using light microscopy and scanning electron microscopy. All the mouthparts, except the mandible but including the mandibular palp, appeared to be supplied with a wide variety of setae. The setae of the medial rims of these appendages (the 'ventral screens') show a trend of decreasing passive motility towards the mouth. This trend is shown by the setae as well as the micro-structures on the setae. The 3rd maxillipeds (MP3) have long simple setae on all segments, the MP2 have a screen of long plumose setae on the meropodites, forming a network with a 30 µm mesh size. The basipodites of the MP1 are densely covered with a mixture of long simple and long pappose setae, both with fine serrulations at the tip. The medial rims of the 2nd maxillae (Mx2) have rows of stiff setae with setules or denticles, and serrated tips. The Mx1 have teeth-like cuspidate setae on the medial rims of the basipodites, the coxopodites bear denticulated setae. The medial rim of the mandibular inscissor process has 11 to 12 tooth-shaped projections, the mandibular palp is distally covered with plumodenticulate setae and with serrated setae with sometimes large scales.

The motion of the mouthparts has been filmed from aboral and from rostral with macro-video and endoscopy equipment, after marking the joints and tips of maxillipeds and the mandibles. Motion analysis showed that all mouthparts except the Mx1 actively moved during deposit feeding. The MP2, MP1, Mx2 and Mandibles moved at the same frequency, contralateral in phase, but with a 50 % phase shift between ipsilateral mouthparts. The MP3 moved at a lower frequency with a contralateral phase shift of about 50 %, without an obvious phase relation with the other mouthparts. Morphology and motion of the appendages were combined with specific setal functions in a synthesis for *C. subterranea's* feeding mechanism. The MP3 make large excursions over the substrate, suspending the sediment in front of the MP2. The MP2 move through the upper layer of the suspension, trapping particles of 30 µm and smaller. These particles are combed out by the MP1 and transferred to the mouth opening by the cascade of finely structured setae of the inner mouthparts, and finally ingested.

E. Quantitative flow analysis on aquatic animals, with *C. subterranea* as one of the examples

Two alternative particle image velocimetry (PIV) methods have been developed, applying laser light sheet illumination of particle seeded flows around marine organisms. Successive video images, recorded perpendicular to a light sheet parallel to the main stream, were digitized and processed to map the flow velocity in 2D planes. In particle tracking velocimetry (PTV), displacements of single particles in two subsequent images were determined semi-automatically resulting in flow diagrams consisting of non-uniformly distributed velocity vectors. Application of grid-cell averaging resulted in flow field diagrams with uniform vector distribution. In sub-image correlation PIV (SCPIV), repetitive convolution filtering of small sub-areas of 2 subsequent images resulted in automatic determination of cross-correlation peaks, yielding flow field diagrams with regularly spaced velocity vectors. In both PTV and SCPIV, missing values, caused by incomplete particle displacement information in some areas of the images or due to rejection of some erroneous vectors by the vector validation procedure, were interpolated using a 2D spline interpolation technique. The resultant vector flow fields were used to study the spatial distribution of velocity, spatial acceleration, vorticity, strain and shear. These flow fields could also be used to test for flow in the third dimension by studying the divergence, and to detect the

presence and location of vortices. The results offer detailed quantitative descriptions of the flow morphology and can be used to assess dissipated energy. The versatile character of the technique makes it applicable to a wide range of fluid mechanical subjects within biological research. So far it has been successfully applied to map the flow around swimming copepods, fish larvae and juvenile fish, and the ventilation current of the tube living shrimp *Callianassa subterranea*.

F. Morphology and motion of the appendages involved in burrow ventilation of *C. subterranea*

The morphology of the pleopods, uropods and telson of *C. subterranea* have been studied applying dissection microscopy and scanning electron microscopy. The kinematics of these appendages has been traced by motion analysis of macro-video recordings of ventilating shrimps in transparent artificial burrows. The pleopods show the common crustacean biramous anatomy, but all segments are rostro-caudally flattened. The protopod bears a triangular medially oriented endopod and a scoop-shaped exopod. The contra-lateral endopods are linked by the 'appendix interna', ensuring a perfect phase relation with no shift between contra-lateral pleopods. The outer rims of the exopods are fringed with long fern-leaf-like plumose setae with flattened setules. These setae have very motile joints and can be turned caudally. The slits between contralateral endopods have rims of leaf setae as well. The same type but non-motile leaf setae are fringing the uropods. The telson has a narrow fringe of leaf setae, locally interrupted by long mechano-receptory setae.
When a shrimp is wandering through the burrow or resting, the pleopods are held against the abdomen with the exopods and their setal fringes retracted to medial. The uropods are folded to medial as well, probably to reduce the shrimps drag. During ventilation the uropods are extended against the tube wall, leaving only a small opening for flow ventral from the telson, and the pleopods beat at a frequency of about 1 Hz (0.9 ± 0.2). Fourier analysis of the pleopod kinematics showed that the motion pattern of the first flow generating pleopod pair (PP1) consisted mainly of the first harmonic (75%) and to a lesser extend of the third harmonic (20%), resulting in an almost perfect sinussoidal motion. The motion patterns of PP2 and PP3 did not yield valid Fourier results, but could be modelled by assigning pure sinussoids with a 120° phase shift and a rostro-caudal ranking to the three pairs of pleopods.

G. The flow in the burrow in the vicinity of a ventilating *C. subterranea*

The flow profiles in front of a ventilating *C. subterranea* in a transparent artificial burrow, around the shrimps abdomen, directly behind its telson and further behind its telson has been studied quantitatively applying particle image velocimetry (PIV). Pulsation of the flow has been studied by tracing single particles in time in the main flow through the burrow. The flow in front of the shrimp showed all the characteristics of normal Hagen-Poisseuille flow: a constant parabolic velocity profile and no pulsation. This flow is split up and accelerated when passing the shrimps cephalothorax and becomes highly pulsating around the beating pleopods. Water masses around and between the pleopods are accelerated by the pleopods and drained off in caudal and ventral direction after being shedded. In the lower part of the (horizontal) tube, the water is mainly accelerated by the extended rims of the pleopods, entering this region during the power stroke only. A converging flow with steeply increasing velocity toward caudal is the net result. The water leaves the pleopod area through the small opening below the telson and is ejected as a jet with an velocity 8 times as high as in front of the shrimp. Due to the steep velocity gradients, a flow separation zone with backflow is found above the jet, behind the telson. Further backwards the jet diverges and decreases, and finally transforms to a parabolic velocity profile within a distance of 2.5 tube diameters (ca. one body length) behind the shrimp. The flow behind the shrimp again shows no pulsation.
C. subterranea apparently manages to avoid pulsation of the flow in its burrow by having inertial forces prevail above viscous forces by increasing the flow velocity due to the tailfan constriction. The energetic advantages of avoiding pulsation are discussed by comparing the hydrodynamics and energetics of a pulsating and a steady flow in a tube. A pulsating flow of the same flow rate would increase the dissipated energy by a factor 6. Avoiding pulsation therefore pays off.

H. Hydrodynamic modelling of pleopod pumping energetics in *C. subterranea*

The process of flow generation with metachronally beating pleopods in a tubiform burrow has been studied by designing a hydrodynamic model based on a thrust-drag force balance. The drag of the tube (including the shrimp) comprises components for accelerating the water into the tube entrance, adjusting a parabolic

velocity profile, accelerating the flow into a constriction due to the body and an additional constriction due to the extended tail fan, shear due to separation, and the viscous resistance of all tube parts. The thrust produced by the beating pleopods comprises components for the drag based thrust and the added mass based thrust. The beating pleopods are approximated by oscillating flat plates with a different area and camber during the power stroke and the recovery stroke, and a phase shift between adjacent pleopods. The added mass is shedded during the second half of the power stroke, and minimized during the recovery stroke. A force balance between the pleopod-thrust and the tube-drag is effected by calculating the average thrust during one beat cycle at a certain flow velocity in the tube and compare it to the drag of the tube at that flow velocity. The energetics of the tube and the pump are derived from the forces, and the mechanical efficiency of the system is the ratio of these two. Adjusted at standard *C. subterranea* settings, the model predicts an average flow velocity in the tube of 1.8 mm/s. The average thrust force, equalling the drag, is 36.8 μN, the work done by the pleopod pump per beat cycle is 0.91 μJ and the dissipated energy by the tube system is 0.066 μJ per cycle. The mechanical efficiency is therefore 7.3 %. The 'pump-parameters' that may be varied by the shrimp are: beat frequency, phase shift, amplitude and the difference in pleopod area between power and recovery stroke. These parameters are varied in the model as well to evaluate their effect. Furthermore, the moment of added mass shedding, the distance between adjacent pleopods, the number of pleopods and the total tube drag were varied to understand and evaluate their effect as well. The process of added mass shedding is discussed in more detail, since this is a new feature in an hydrodynamic model for paddling structures. Given the constraints for creating a steady flow in a tube, *C. subterranea* seems a reasonably optimal pump, as far as this model can evaluate.

I. Behavioural respiratory energetics of *C. subterranea*

Individual *C. subterranea* were subjected to recirculation respirometry meanwhile recording their behaviour, to correlate respiration levels to differences in behaviour. To ensure a minimal system respiration and reduce sources of error, a number of precautions were taken. The shrimps were washed with sterile seawater and starved in a respirometer-like environment prior to the experiments, to reduce adhering bacteria and minimize digestion metabolism. The respirometer was designed as a burrow-like transparent U-tube with two turning chambers, enabling the shrimp to

display at least some of its natural behaviour. The respirometer and the cooling basin contained continuously sterilized and filtered water, and the whole setup was electrically insulated from all the electronic equipment, except for the oxygen probe. Five experiments of 9 hours of continuously recording the oxygen concentration in the respirometer and recording the behaviour were realized, each preceded by an additional 1 hr acclimatization period. The results were split into 45 one-hr respiration levels and time budgets. Most of the behavioural classes found in natural behaviour were also found in the behaviour of the shrimps in the respirometer.
The respirometry levels showed a significant relation with animal ash-free dry mass (AFDM). The data were compensated for effects of mass, and were correlated to the separate behavioural states. Respiration decreased with an increasing percentage of time resting, and consequently respiration increased with increasing total activity. The increase in respiration with total activity is mainly caused by an increase in respiration with increasing time spent ventilating. Respiratory equivalents per behavioural state were derived, leading to 2 functional classes in respiratory costs (in $mgO_2/hr/gAFDM$ ± SD) for an average size shrimp (AFDM = 0.086 mg) with respect to behaviour: Activity + Resting = 0.083 ± 0.016 , and Ventilating = 0.126 ± 0.024 . Average respiration levels constructed from *C. subterranea's* time budget and the energetic equivalents happened to be the same as the average levels in the respirometer. The mass-specific respiration as a function of AFDM when displaying natural behaviour therefore is: $R^*_M = (0.040 \pm 0.010) * AFDM^{(-0.30 \pm 0.12)}$ in $mgO_2/hr/gAFDM$. Oxycaloric conversion results in an expression for mass-specific active metabolic rate of *C. subterranea* as a function of AFDM: $AMR_M = (0.15 \pm 0.05) * AFDM^{(-0.30 \pm 0.12)}$ in mW/gAFDM. A comparisson of the additional metabolic rate for Ventilating with the former results on physical modelling of burrow ventilation show a 4 times lower prediction from modelling. This indicates that modelling may result in predictions of the right order of magnitude, but these predictions should not directly be converted to metabolic energy. A comparison of the respirometry results with former results on food intake energetics shows a difference of a factor 3, which, considering the error margins, may be interpreted as a reasonable match.